Serial No. 09/982,464
Docket No. X-16755A

Amendments to Claims

1. (Currently amended) A method of constructing a population of altered heavy chain variable region encoding nucleic acids, comprising:

A) providing visual representations of first and second reference amino acid sequences, said first reference sequence comprising the sequence of a donor heavy chain variable region, said donor variable region comprising i) non-human donor framework regions and ii) three non-human donor complementarity-determining regions as defined by the combined definitions of Kabat and Chothia; said second reference sequence comprising the sequence of an acceptor heavy chain variable region comprising human acceptor framework regions;

B) synthesizing [[a)]] i) a population of first oligonucleotides encoding portions of said human acceptor framework regions of said acceptor heavy chain variable region ~~to create encoded portions~~ to create encoded portions, wherein said encoded portions of said human acceptor framework regions when compared to said second reference sequence are unmodified; and [[b)]] ii) a population of second oligonucleotides, encoding [[i)]] at least a portion of a first complementarity-determining region s selected from the group consisting of HCDR1, HCDR2 and HCDR3 which has been modified when compared to the non-human donor complementarity-determining regions of said first reference ~~that has been modified so as to create a modified first complementarity-determining region, said first complementarity-determining region selected from the group consisting of HCDR1, HCDR2 and HCDR3, wherein said modified first complementarity-determining region comprises a different amino acid at one or more positions when compared to the corresponding non-human donor complementarity-determining regions of said first reference sequence and ii) one or more portions of unmodified framework regions~~ said population of second oligonucleotides comprising at least portions which are capable of hybridizing to said first oligonucleotides;

C) mixing said population of first oligonucleotides with said population of second oligonucleotides so as to create overlapping oligonucleotides; and

D) treating said overlapping oligonucleotides under conditions such that a population of ~~altered heavy chain variable region encoding nucleic acids is constructed, wherein the human acceptor framework regions encoded by said altered heavy chain variable region encoding nucleic acids are unmodified with respect to said second reference sequence.~~ nucleic acids encoding heavy chain variable regions comprising unmodified framework regions and at least one modified CDR region is constructed.

2. (Cancelled)

3. (Currently amended) The method of Claim 1, further comprising the step of (E) coexpressing said population of ~~altered~~ heavy chain variable region encoding nucleic acids with a light chain variable region encoding nucleic acid so as to produce a diverse population of altered heteromeric variable regions.

4. (Cancelled)

5. (Cancelled)

6. (Cancelled)

7. (Currently amended) A method of constructing a population of altered light chain variable region encoding nucleic acids, comprising:

A) providing visual representations of first and second reference amino acid sequences, said first reference sequence comprising the sequence of a donor light chain variable region, said donor variable region comprising i) non-human donor framework regions and ii) three non-human donor complementarity-determining regions as defined by the combined definitions of Kabat and Chothia; said second reference sequence comprising the sequence of an acceptor light chain variable region comprising human acceptor framework regions;

B) synthesizing [[a]] i) a population of first oligonucleotides encoding portions of said human acceptor framework regions of said acceptor light chain

variable region ~~to create encoded portions~~, wherein said encoded portions of said human acceptor framework regions when compared to said second reference sequence are unmodified; and [[b)]] ii) a population of second oligonucleotides, each encoding ii) at least a portion of a first complementarity determining region selected from the group consisting of LCDRI, LCDR2 and LCDR3 which has been modified when compared to the non-human donor complementarity-determining regions of said first reference ~~that has been modified so as to create a modified first complementarity determining region, said first complementarity determining region selected from the group consisting of LCDRI, LCDR2 and LCDR3, wherein said modified first complementarity determining region comprises a different amino acid at one or more positions when compared to the corresponding non-human donor complementarity determining regions of said first reference sequence and ii) one or more portions of unmodified framework regions~~ said population of second oligonucleotides comprising at least portions which are capable of hybridizing to said first oligonucleotides;

C) mixing said first oligonucleotides with said population of second oligonucleotides as to create overlapping oligonucleotides; and

D) treating said overlapping oligonucleotides under conditions such that a population of ~~altered light chain variable region encoding nucleic acids is constructed, wherein the human acceptor framework regions encoded by said altered light chain variable region encoding nucleic acids are unmodified with respect to said second reference sequence.~~ nucleic acids encoding light chain variable regions comprising unmodified framework regions and at least one modified CDR region is constructed.

8. (Cancelled)

9. (Currently amended) The method of Claim 7, further comprising the step of (E) coexpressing said population of ~~altered~~ light chain variable region encoding nucleic acids with a heavy chain variable region encoding nucleic acid so as to produce a diverse population of altered heteromeric variable regions.

10. (Cancelled)

11. (Cancelled)

12. (Cancelled)

13. (Currently amended) A method of constructing a population of altered heavy chain variable region encoding nucleic acids, comprising:

A) providing visual representations of first and second reference amino acid sequences, said first reference sequence comprising the sequence of a donor heavy chain variable region, said donor variable region comprising i) non-human donor framework regions and ii) three non-human donor complementarity-determining regions as defined by the combined definitions of Kabat and Chothia; said second reference sequence comprising the sequence of an acceptor heavy chain variable region comprising human acceptor framework regions;

B) synthesizing [[a]] i) a population of first oligonucleotides, ~~each~~ encoding at least a portion of a first complementarity-determining region selected from the group consisting of HCDR1, HCDR2 and HCDR3 which ~~that~~ has been modified ~~so as to create a modified first complementarity determining region, said first complementarity determining region selected from the group consisting of HCDR1, HCDR2 and HCDR3, wherein said modified first complementarity determining region comprises a different amino acid at one or more positions~~ when compared to the ~~corresponding~~ non-human donor complementarity determining regions of said first reference sequence; and [[b]] ii) a population of second oligonucleotides encoding [[i)]] portions of said human acceptor framework regions, ~~of said acceptor heavy chain variable regions to create encoded portions,~~ wherein said encoded portions of said human acceptor framework regions are unmodified when compared to said second reference sequence ~~are unmodified~~, said population of second oligonucleotides comprising at least portions ~~and ii) one or more portions of a complementarity determining region~~ which are capable of hybridizing to said population of first oligonucleotides;

C) mixing said population of first oligonucleotides with said second oligonucleotides as to create overlapping oligonucleotides; and

D) treating said overlapping oligonucleotides under conditions such that a population of ~~altered heavy chain variable region encoding nucleic acids is constructed, wherein the human acceptor framework regions encoded by said altered heavy chain variable region encoding nucleic acids are unmodified with respect to said second reference sequence.~~ nucleic acids encoding heavy chain variable regions comprising unmodified framework regions and at least one modified CDR region is constructed.

14. (Cancelled)

15. (Currently amended) The method of Claim 13, further comprising the step of (E) coexpressing said population of ~~altered~~ heavy chain variable region encoding nucleic acids with a light chain variable region encoding nucleic acid so as to produce a diverse population of altered heteromeric variable regions.

16. (Cancelled)

17. (Cancelled)

18. (Cancelled).

19. (Currently amended) A method of constructing a population of altered light chain variable region encoding nucleic acids, comprising:

A) providing visual representations of first and second reference amino acid sequences, said first reference sequence comprising the sequence of a donor light chain variable region, said donor variable region comprising i) non-human donor framework regions and ii) three non-human donor complementarity-determining regions as defined by the combined definitions of Kabat and Chothia; said second reference sequence comprising the sequence of an acceptor light chain variable region comprising human acceptor framework regions;

B) synthesizing [[a)]] i) a population of first oligonucleotides, ~~each~~ encoding at least a portion of a first complementarity-determining region selected from the group consisting of LCDRI, LCDR2 and LCDR3, which ~~that~~ has been modified ~~so as to create a modified first complementarity-determining region, said first complementarity-determining region selected from the group consisting of LCDRI, LCDR2 and LCDR3, wherein said modified first complementarity-determining region comprises a different amino acid at one or more positions~~ when compared to the ~~corresponding~~ non-human donor complementarity determining regions of said first reference sequence; and [[b]] ii) a population of second oligonucleotides encoding ~~i)~~ i) portions of said human acceptor framework regions, ~~of said acceptor light chain variable regions to create encoded portions,~~ wherein said encoded portions of said human acceptor framework regions are unmodified when compared to said second reference sequence ~~are unmodified~~, said population of second oligonucleotides comprising at least portions ~~and ii) one or more portions of a complementarity determining region~~ which are capable of hybridizing to said population of first oligonucleotides;

C) mixing said population of first oligonucleotides with said second oligonucleotides as to create overlapping oligonucleotides; and

D) treating said overlapping oligonucleotides under conditions such that a population of ~~altered light chain variable region encoding nucleic acids is constructed, wherein the human acceptor framework regions encoded by said altered light chain variable region encoding nucleic acids are unmodified with respect to said second reference sequence.~~ nucleic acids encoding light chain variable regions comprising unmodified framework regions and at least one modified CDR region is constructed.

20. (Cancelled)

21. (Currently amended) The method of Claim 19, further comprising the step of (E) coexpressing said population of ~~altered~~ light chain variable region encoding nucleic acids with a heavy chain variable region encoding nucleic acid so as to produce a diverse population of altered heteromeric variable regions.

22. (Cancelled)

23. (Cancelled)

24. (Cancelled)

25. (New) The method of claim 1 wherein, said population of nucleic acids encoding heavy chain variable regions of step (D) comprises at least two modified complementarity-determining regions.

26. (New) The method of claim 1 wherein said population of nucleic acids encoding heavy chain variable regions of step (D) comprises at least three modified complementarity-determining regions.

27. (New) The method of Claim 25, further comprising the step of (E) coexpressing said population of nucleic acids encoding heavy chain variable regions with nucleic acids encoding the light chain variable regions so as to produce a diverse population of altered heteromeric variable regions.

28. (New) The method of Claim 26, further comprising the step of (E) coexpressing said population of nucleic acids encoding heavy chain variable regions with nucleic acids encoding a light chain variable region so as to produce a diverse population of altered heteromeric variable regions.

29. (New) The method of claim 7 wherein, said population of nucleic acids encoding light chain variable regions of step (D) comprises at least two modified complementarity-determining regions.

30. (New) The method of claim 7 wherein said population of nucleic acids encoding light chain variable regions of step (D) comprises at least three modified complementarity-determining regions.

31. (New) The method of Claim 29 further comprising the step of (E) coexpressing said population of nucleic acids encoding light chain variable regions with nucleic acids encoding a heavy chain variable region so as to produce a diverse population of altered heteromeric variable regions.

32. (New) The method of Claim 30 further comprising the step of (E) coexpressing said population of nucleic acids encoding light chain variable regions with nucleic acids encoding a heavy chain variable region so as to produce a diverse population of altered heteromeric variable regions.

33. (New) The method of claim 13 wherein said population of nucleic acids of step (D) comprises at least two modified complementarity-determining regions.

34. (New) The method of claim 13 wherein said population of nucleic acids of step (D) comprises at least three modified complementarity-determining regions.

35. (New) The method of Claim 33 further comprising the step of (E) coexpressing said population of nucleic acids encoding heavy chain variable regions with nucleic acids encoding a light chain variable region so as to produce a diverse population of altered heteromeric variable regions.

36. (New) The method of Claim 34 further comprising the step of (E) coexpressing said population of nucleic acids encoding heavy chain variable regions with nucleic acids encoding a light chain variable region so as to produce a diverse population of altered heteromeric variable regions.

37. (New) The method of claim 19 wherein, said population of nucleic acids of step (D) comprises at least two modified complementarity-determining regions.

38. (New) The method of claim 19 wherein said population of nucleic acids of step (D) comprises at least three modified complementarity-determining regions.

39. (New) The method of Claim 37 further comprising the step of (E) coexpressing said population of nucleic acids encoding light chain variable regions with nucleic acids encoding a heavy chain variable region so as to produce a diverse population of altered heteromeric variable regions.

40. (New) The method of Claim 38 further comprising the step of (E) coexpressing said population of nucleic acids encoding light chain variable regions with nucleic acids encoding a heavy chain variable region so as to produce a diverse population of altered heteromeric variable regions.

41. (New) A method of constructing a population of antibody variable regions comprising:

A) constructing a population of nucleic acids encoding heavy chain variable regions comprising:

i) providing visual representations of first and second reference amino acid sequences, said first reference sequence comprising the sequence of a donor heavy chain variable region, said donor variable region comprising a) non-human donor framework regions and b) three non-human donor complementarity-determining regions as defined by the combined definitions of Kabat and Chothia; said second reference sequence comprising the sequence of an acceptor heavy chain variable region comprising human acceptor framework regions;

ii) synthesizing a) a population of first oligonucleotides, encoding at least a portion of a first complementarity-determining region selected from the group consisting of HCDRI, HCDR2 and HCDR3, which has been modified when compared to the non-human donor complementarity determining region of said first reference sequence; and b) a population of second oligonucleotides encoding portions of said human acceptor framework regions, wherein said encoded portions of said human acceptor framework regions are unmodified when compared to said second

reference sequence, said population of second oligonucleotides comprising at least portions which are capable of hybridizing to said population of first oligonucleotides;

iii) mixing said population of first oligonucleotides with said second oligonucleotides as to create overlapping oligonucleotides; and

iv) treating said overlapping oligonucleotides under conditions such that a population of nucleic acids encoding light chain variable regions comprising unmodified framework regions and at least one modified CDR regions is constructed;

B) constructing a population of nucleic acids encoding light chain variable regions comprising:

i) providing visual representations of third and fourth reference amino acid sequences, said third reference sequence comprising the sequence of a donor light chain variable region, said donor variable region comprising a) non-human donor framework regions and b) three non-human donor complementarity-determining regions as defined by the combined definitions of Kabat and Chothia; said fourth reference sequence comprising the sequence of an acceptor light chain variable region comprising human acceptor framework regions;

ii) synthesizing a) a population of first oligonucleotides, encoding at least a portion of a first complementarity-determining region selected from the group consisting of LCDRI, LCDR2 and LCDR3, which has been modified when compared to the non-human donor complementarity determining region of said first reference sequence; and b) a population of second oligonucleotides encoding portions of said human acceptor framework regions, wherein said encoded portions of said human acceptor framework regions are unmodified when compared to said second reference sequence, said population of second oligonucleotides comprising at least portions which are capable of hybridizing to said population of first oligonucleotides;

iii) mixing said population of first oligonucleotides with said second oligonucleotides as to create overlapping oligonucleotides; and

iv) treating said overlapping oligonucleotides under conditions such that a population of nucleic acids encoding light chain variable regions comprising unmodified framework regions and at least one modified CDR regions is constructed.

C) coexpressing said population of nucleic acids encoding heavy chain variable regions with said population of nucleic acids encoding light chain variable regions to produce an

antibody variable region comprising unmodified heavy chain and light chain frameworks and at least one modified heavy chain complementarity-determining region and at least one modified light chain complementarity-determining region.

42. (New) The method of claim 41 wherein said antibody variable region further comprises at least three modified complementarity-determining regions.

43. (New) The method of claim 41 wherein said antibody variable region further comprises at least four modified complementarity-determining regions.

44. (New) The method of claim 41 wherein said antibody variable region further comprises at least five modified complementarity-determining regions.

45. (New) The method of claim 41 wherein said antibody variable region further comprises six modified complementarity-determining regions.